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(54) Title: METHOD OF PREPARING A HEAT-TREATED PRODUCT

(57) Abstract: The formation of acrylamide during heat treatment in the production of a food product is reduced by treating the raw material with an enzyme before the heat treatment. The enzyme is capable of reacting on asparagine or glutamine (optionally substituted) as a substrate or is a laccase or a peroxidase.

METHOD OF PREPARING A HEAT-TREATED PRODUCT

FIELD OF THE INVENTION

The present invention relates to a method of preparing a heat-treated product with a low water content from raw material comprising carbohydrate, protein and water. It also relates to an asparaginase for use in the method

BACKGROUND OF THE INVENTION

E. Tabeke et al. (*J. Agric. Food Chem.*, 2002, 50, 4998-5006) reported that acrylamide is formed during heating of starch-rich foods to high temperatures. The acrylamide formation has been ascribed to the Maillard reaction (D.S. Mottram et al., R.H. Stadler et al., *Nature*, 419, 3 October 2002, 448-449).

WO 00/56762 discloses expressed sequence tags (EST) from *A. oryzae*.

Kim, K.-W.; Kamerud, J.Q.; Livingston, D.M.; Roon, R.J., (1988) Asparaginase II of *Saccharomyces cerevisiae*. Characterization of the ASP3 gene. *J. Biol. Chem.* 263:11948, discloses the peptide sequence of an extra-cellular asparaginase

SUMMARY OF THE INVENTION

According to the invention, the formation of acrylamide during heat treatment of raw material comprising carbohydrate, protein and water is reduced by treating the raw material with an enzyme before the heat treatment. Accordingly, the invention provides a method of preparing a heat-treated product, comprising the sequential steps of:

- a) providing a raw material which comprises carbohydrate, protein and water
- b) treating the raw material with an enzyme, and
- c) heat treating to reach a final water content below 35 % by weight.

The enzyme is capable of reacting on asparagine or glutamine (optionally substituted) as a substrate or is a laccase or a peroxidase.

The invention also provides an asparaginase for use in the process and a polynucleotide encoding the asparaginase.

DETAILED DESCRIPTION OF THE INVENTION

Raw material and enzyme treatment

The raw material comprises carbohydrate, protein and water, typically in amounts of 10-90 % or 20-50 % carbohydrate of the total weight. The carbohydrate may consist mainly of starch, and it may include reducing sugars such as glucose, e.g. added as glucose syrup,

honey or dry dextrose. The protein may include free amino acids such as asparagine and glutamine (optionally substituted).

The raw material may include tubers, potatoes, grains, oats, barley, corn (maize), wheat, nuts, fruits, dried fruit, bananas, sesame, rye and/or rice.

5 The raw material may be in the form of a dough comprising finely divided ingredients (e.g. flour) with water. The enzyme treatment may be done by mixing (kneading) the enzyme into the dough and optionally holding to let the enzyme act. The enzyme may be added in the form of an aqueous solution, a powder, a granulate or agglomerated powder. The dough may be formed into desired shapes, e.g. by sheeting, cutting and/or extrusion.

10 The raw material may also be in the form of intact vegetable pieces, e.g. slices or other pieces of potato, fruit or bananas, whole nuts, whole grains etc. The enzyme treatment may comprise immersing the vegetable pieces in an aqueous enzyme solution and optionally applying vacuum infusion. The intact pieces may optionally be blanched by immersion in hot water, e.g. at 70-100°C, either before or after the enzyme treatment.

15 The raw material may be grain intended for malting, e.g. malting barley or wheat. The enzyme treatment of the grain may be done before, during or after the malting (germination).

The raw material before heat treatment typically has a water content of 10-90 % by weight and is typically weakly acidic, e.g. having a pH of 5-7.

Heat treatment

20 The process of the invention involves a heat treatment at high temperature to reach a final water content (moisture content) in the product below 35 % by weight, typically 1-20 %, 1-10 % or 2-5 %. During the heat treatment, the temperature at the surface of the product may reach 110-220°C, e.g. 110-170°C or 120-160°C.

25 The heat treatment may involve, frying, particularly deep frying in tri- and/or di-glycerides (animal or vegetable oil or fat, optionally hydrogenated), e.g. at temperatures of 150-180°C. The heat treatment may also involve baking in hot air, e.g. at 160-310°C or 200-250°C for 2-10 minutes, or hot-plate heating. Further, the heat treatment may involve kilning of green malt.

Heat-treated product

30 The process of the invention may be used to produce a heat-treated product with low water content from raw material containing carbohydrate and protein, typically starchy food products fried or baked at high temperatures. The heat-treated product may be consumed directly as an edible product or may be used as an ingredient for further processing to prepare an edible or potable product.

Examples of products to be consumed directly are potato products, potato chips (crisps), French fries, hash browns, roast potatoes, breakfast cereals, crisp bread, muesli, biscuits, crackers, snack products, tortilla chips, roasted nuts, rice crackers (Japanese "senbei"), wafers, waffles, hot cakes, and pancakes.

- 5 Malt (e.g. caramelized malt or so-called chocolate malt) is generally further processed by mashing and brewing to make beer.

Enzyme capable of reacting with asparagine or glutamine (optionally substituted) as a substrate

- The enzyme may be capable of reacting with asparagine or glutamine which is optionally glycosylated or substituted with a peptide at the alpha-amino and/or the carboxyl position. The enzyme may be an asparaginase, a glutaminase, an L-amino acid oxidase, a glycosylasparaginase, a glycoamidase or a peptidoglutaminase.

- The glutaminase (EC 3.5.1.2) may be derived from *Escherichia coli*. The L-amino acid oxidase (EC 1.4.3.2) capable of reacting with asparagine or glutamine (optionally glycosylated) as a substrate may be derived from *Trichoderma harzianum* (WO 94/25574). The glycosylasparaginase (EC 3.5.1.26, aspartylglucosaminidase, N4-(N-acetyl-beta-glucosaminyl)-L-asparagine amidase) may be derived from *Flavobacterium meningosepticum*. The glycoamidase (peptide N-glycosidase, EC 3.5.1.52) may be derived from *Flavobacterium meningosepticum*. The peptidoglutaminase may be peptidoglutaminase I or II (EC 3.5.1.43, EC 3.5.1.44).

- 20 The enzyme is used in an amount which is effective to reduce the amount of acrylamide in the final product. The amount may be in the range 0.1-100 mg enzyme protein per kg dry matter, particularly 1-10 mg/kg. Asparaginase may be added in an amount of 10-100 units per kg dry matter where one unit will liberate 1 micromole of ammonia from L-asparagine per min at pH 8.6 at 37 °C

25 Asparaginase

- The asparaginase (EC 3.5.1.1) may be derived from *Saccharomyces cerevisiae*, *Candida utilis*, *Escherichia coli*, *Aspergillus oryzae*, *Aspergillus nidulans*, *Aspergillus fumigatus*, *Fusarium graminearum*, or *Penicillium citrinum*. It may have the amino acid sequence shown in SEQ ID NO: 2 (optionally truncated to residues 27-378, 30-378, 75-378 or 80-378), 4, 6, 8, 10, 12 or 13 or a sequence which is at least 90 % (particularly at least 95 %) identical to one of these. It may be produced by use of the genetic information in SEQ ID NO: 1, 3, 5, 7, 9 or 11, e.g., as described in an example.

- Whitehead Institute, MIT Center for Genome Research, Fungal Genome Initiative has published *A. nidulans* release 1 and *F. graminearum* release 1 on the Internet at <http://www-genome.wi.mit.edu/ftp/distribution/annotation/> under the *Aspergillus* Sequencing Project and

the *Fusarium graminearum* Sequencing Project. Preliminary sequence data for *Aspergillus fumigatus* was published on The Institute for Genomic Research website at <http://www-genome.wi.mit.edu/ftp/distribution/annotation/>.

The inventors inserted the gene encoding the asparaginase from *A. oryzae* into *E. coli* and deposited the clone under the terms of the Budapest Treaty with the DSMZ - Deutsche Sammlung von Microorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig. The deposit number was DSM 15960, deposited on 6 October 2003.

Alignment and identity

The enzyme and the nucleotide sequence of the invention may have homologies to the disclosed sequences of at least 90 % or at least 95 %, e.g. at least 98 %.

For purposes of the present invention, alignments of sequences and calculation of identity scores were done using a Needleman-Wunsch alignment (i.e. global alignment), useful for both protein and DNA alignments. The default scoring matrices BLOSUM50 and the identity matrix are used for protein and DNA alignments respectively. The penalty for the first residue in a gap is -12 for proteins and -16 for DNA, while the penalty for additional residues in a gap is -2 for proteins and -4 for DNA. Alignment is from the FASTA package version v20u6 (W. R. Pearson and D. J. Lipman (1988), "Improved Tools for Biological Sequence Analysis", PNAS 85:2444-2448, and W. R. Pearson (1990) "Rapid and Sensitive Sequence Comparison with FASTP and FASTA", Methods in Enzymology, 183:63-98).

Laccase or peroxidase

The laccase (EC 1.10.3.2) may be of plant or microbial origin, e.g. from bacteria or fungi (including filamentous fungi and yeasts). Examples include laccase from *Aspergillus*, *Neurospora*, e.g., *N. crassa*, *Podosporea*, *Botrytis*, *Collybia*, *Fomes*, *Lentinus*, *Pleurotus*, *Trametes*, e.g., *T. villosa* and *T. versicolor*, *Rhizoctonia*, e.g., *R. solani*, *Coprinus*, e.g., *C. cinereus*, *C. comatus*, *C. friesii*, and *C. plicatilis*, *Psathyrella*, e.g., *P. condelleana*, *Panaeolus*, e.g., *P. papilionaceus*, *Myceliophthora*, e.g., *M. thermophila*, *Schytalidium*, e.g., *S. thermophilum*, *Polyporus*, e.g., *P. pinsitus*, *Phlebia*, e.g., *P. radita*, or *Coriolus*, e.g., *C. hirsutus*.

The peroxidase (EC 1.11.1.7) may be from plants (e.g. horseradish or soybean peroxidase) or microorganisms such as fungi or bacteria, e.g. *Coprinus*, in particular *Coprinus cinereus* f. *microsporus* (IFO 8371), or *Coprinus macrorhizus*, *Pseudomonas*, e.g. *P. fluorescens* (NRRL B-11), *Streptovorticillium*, e.g. *S. verticillium* ssp. *verticillium* (IFO 13864), *Streptomyces*, e.g. *S. thermoviolaceus* (CBS 278.66), *Streptomyces*, e.g. *S. viridosporus* (ATCC 39115), *S. badius* (ATCC 39117), *S. phaeochromogenes* (NRRL B-3559), *Pseudomonas*, e.g. *P. pyrocinia* (ATCC 15958), *Fusarium*, e.g. *F. oxysporum* (DSM 2672) and *Bacillus*, e.g. *B. stearothermophilus* (ATCC 12978).

Oxidoreductase capable of reacting with a reducing sugar as a substrate

The method of the invention may comprise treating the raw material with an oxidoreductase capable of reacting with a reducing sugar as a substrate. The oxidoreductase may be an oxidase or dehydrogenase capable of reacting with a reducing sugar as a substrate such as
5 glucose and maltose.

The oxidase may be a glucose oxidase, a pyranose oxidase, a hexose oxidase, a galactose oxidase (EC 1.1.3.9) or a carbohydrate oxidase which has a higher activity on maltose than on glucose. The glucose oxidase (EC 1.1.3.4) may be derived from *Aspergillus niger* e.g. having the amino acid sequence described in US 5094951. The hexose oxidase (EC 1.1.3.5)
10 may be derived from algal species such as *Iridophycus flaccidum*, *Chondrus crispus* and *Euthora cristata*. The pyranose oxidase may be derived from *Basidiomycete* fungi, *Peniophora gigantean*, *Aphylophorales*, *Phanerochaete chrysosporium*, *Polyporus pinsitus*, *Bierkandera adusta* or *Phlebiopsis gigantean*. The carbohydrate oxidase which has a higher activity on maltose than on glucose may be derived from *Microdochium* or *Acremonium*, e.g. from *M. nivale*
15 (US 6165761), *A. strictum*, *A. fusidioides* or *A. potronii*.

The dehydrogenase may be glucose dehydrogenase (EC 1.1.1.47, EC 1.1.99.10), galactose dehydrogenase (EC 1.1.1.48), D-aldohexose dehydrogenase (EC 1.1.1.118, EC 1.1.1.119), cellobiose dehydrogenase (EC 1.1.5.1, e.g. from *Humicola insolens*), fructose dehydrogenase (EC 1.1.99.11, EC 1.1.1.124, EC 1.1.99.11), aldehyde dehydrogenase (EC
20 1.2.1.3, EC 1.2.1.4, EC 1.2.1.5). Another example is glucose-fructose oxidoreductase (EC 1.1.99.28).

The oxidoreductase is used in an amount which is effective to reduce the amount of acrylamide in the final product. For glucose oxidase, the amount may be in the range 50-20,000 (e.g. 100-10,000 or 1,000-5,000) GODU/kg dry matter in the raw material. One GODU
25 is the amount of enzyme which forms 1 μ mol of hydrogen peroxide per minute at 30°C, pH 5.6 (acetate buffer) with glucose 16.2 g/l (90 mM) as substrate using 20 min. incubation time. For other enzymes, the dosage may be found similarly by analyzing with the appropriate substrate.

EXAMPLES

Media

30 DAP2C-1

11g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

1g KH_2PO_4

2g Citric acid, monohydrate

30g maltodextrin

6g $K_3PO_4 \cdot 3H_2O$

0.5g yeast extract

0.5ml trace metals solution

1ml Pluronic PE 6100 (BASF, Ludwigshafen, Germany)

- 5 Components are blended in one liter distilled water and portioned out to flasks, adding 250 mg $CaCO_3$ to each 150ml portion.

The medium is sterilized in an autoclave. After cooling the following is added to 1 liter of medium:

23 ml 50% w/v $(NH_4)_2HPO_4$, filter sterilized

- 10 33 ml 20% lactic acid, filter sterilized

Trace metals solution

6.8g $ZnCl_2$

2.5g $CuSO_4 \cdot 5H_2O$

0.24g $NiCl_2 \cdot 6H_2O$

- 15 13.9g $FeSO_4 \cdot 7H_2O$

8.45g $MnSO_4 \cdot H_2O$

3g Citric acid, monohydrate

Components are blended in one liter distilled water.

Asparaginase activity assay

20 *Stock solutions*

50 mM Tris buffer, pH 8.6

189mM L-Asparagine solution

1.5 M Trichloroacetic Acid (TCA)

Nessler's reagent, Aldrich Stock No. 34,514-8 (Sigma-Aldrich, St. Louis, Mo. USA)

- 25 Asparaginase, Sigma Stock No. A4887 (Sigma-Aldrich, St. Louis, Mo. USA)

Assay

Enzyme reaction:

500 micro-l buffer

100 micro-l L-asparagine solution

- 30 350 micro-l water

are mixed and equilibrated to 37 °C.

100 micro-l of enzyme solution is added and the reactions are incubated at 37 °C for 30 minutes.

The reactions are stopped by placing on ice and adding 50 micro-l of 1.5M TCA.

The samples are mixed and centrifuged for 2 minutes at 20,000 g

Measurement of free ammonium:

50 micro-l of the enzyme reaction is mixed with 100 micro-l of water and 50 micro-l of Nessler's reagent. The reaction is mixed and absorbance at 436nm is measured after 1 minute.

Standard:

The asparaginase stock (Sigma A4887) is diluted 0.2, 0.5, 1, 1.5, 2, and 2.5 U/ml.

Example 1: Expression of an asparaginase from *Aspergillus oryzae* in *Aspergillus oryzae*

10 Libraries of cDNA of mRNA from *Aspergillus oryzae* were generated, sequenced and stored in a computer database as described in WO 00/56762.

The peptide sequence of asparaginase II from *Saccharomyces cerevisiae* (Kim,K.-W.; Kamerud,J.Q.; Livingston,D.M.; Roon,R.J., (1988) Asparaginase II of *Saccharomyces cerevisiae*. Characterization of the ASP3 gene. J. Biol. Chem. 263:11948), was compared to translations of the *Aspergillus oryzae* partial cDNA sequences using the TFASTXY program, version 3.2t07 (Pearson et al, Genomics (1997) 46:24-36). One translated *A. oryzae* sequence was identified as having 52% identity to yeast asparaginase II through a 165 amino acid overlap. The complete sequence of the cDNA insert of the corresponding clone (deposited as DSM 15960) was determined and is presented as SEQ ID NO: 1, and the peptide translated from this sequence, AoASP, is presented as SEQ ID NO: 2. This sequence was used to design primers for PCR amplification of the AoASP encoding-gene from DSM 15960, with appropriate restriction sites added to the primer ends to facilitate sub-cloning of the PCR product (primers AoASP7 and AoASP8, SEQ ID NOS: 14 and 15). PCR amplification was performed using Extensor Hi-Fidelity PCR Master Mix (ABgene, Surrey, U.K.) following the manufacturer's instructions and using an annealing temperature of 55°C for the first 5 cycles and 65°C for an additional 30 cycles and an extension time of 1.5 minutes.

The PCR fragment was restricted with *Bam*HI and *Hind*III and cloned into the *Aspergillus* expression vector pMStr57 using standard techniques. The expression vector pMStr57 contains the same elements as pCaHj483 (WO 98/00529), with minor modifications made to the *Aspergillus* NA2 promoter as described for the vector pMT2188 in WO 01/12794, and has sequences for selection and propagation in *E. coli*, and selection and expression in *Aspergillus*. Specifically, selection in *Aspergillus* is facilitated by the *amdS* gene of *Aspergillus nidulans*, which allows the use of acetamide as a sole nitrogen source. Expression in *Aspergillus* is mediated by a modified neutral amylase II (NA2) promoter from *Aspergillus niger* which is fused to the 5' leader sequence of the triose phosphate isomerase (*tpi*) encoding-gene from

Aspergillus nidulans, and the terminator from the amyloglucosidase-encoding gene from *Aspergillus niger*. The asparaginase-encoding gene of the resulting *Aspergillus* expression construct, pMStr90, was sequenced and the sequence agreed completely with that determined previously for the insert of DSM 15960

- 5 The *Aspergillus oryzae* strain BECh2 (WO 00/39322) was transformed with pMStr90 using standard techniques (Christensen, T. et al., (1988), Biotechnology 6, 1419-1422). Transformants were cultured in DAP2C-1 medium shaken at 200 RPM at 30°C and expression of AoASP was monitored by SDS-PAGE and by measuring enzyme activity.

Example 2: Purification of Asparaginase

- 10 Culture broth from the preceding example was centrifuged (20000 x g, 20 min) and the supernatants were carefully decanted from the precipitates. The combined supernatants were filtered through a Seitz EKS plate in order to remove the rest of the *Aspergillus* host cells. The EKS filtrate was transferred to 10 mM Tris/HCl, pH 8 on a G25 sephadex column and applied to a Q sepharose HP column equilibrated in the same buffer. After washing the Q sepharose HP column extensively with the equilibration buffer, the asparaginase was eluted with a
15 linear NaCl gradient (0 --> 0.5M) in the same buffer. Fractions from the column were analysed for asparaginase activity (using the pH 6.0 Universal buffer) and fractions with activity were pooled. Ammonium sulfate was added to the pool to 2.0M final concentration and the pool was applied to a Phenyl Toyopearl S column equilibrated in 20 mM succinic acid, 2.0M (NH₄)₂SO₄,
20 pH 6.0. After washing the Phenyl column extensively with the equilibration buffer, the enzyme was eluted with a linear (NH₄)₂SO₄ gradient (2.0 --> 0M) in the same buffer. Fractions from the column were again analysed for asparaginase activity and active fractions were further analysed by SDS-PAGE. Fractions, which was judged only to contain the asparaginase, were pooled as the purified preparation and was used for further characterization. The purified as-
25 paraginase was heterogeneously glycosylated judged from the coomassie stained SDS-PAGE gel and in addition N-terminal sequencing of the preparation revealed that the preparation contained different asparaginase forms, as four different N-termini were found starting at amino acids A₂₇, S₃₀, G₇₅ and A₈₀ respectively of SEQ ID NO: 2. However, the N-terminal sequencing also indicated that the purified preparation was relatively pure as no other N-terminal se-
30 quences were found by the analysis.

Example 3: Properties of asparaginase

The purified asparaginase from the preceding example was used for characterization.

Asparaginase assay

- A coupled enzyme assay was used. Asparaginase was incubated with asparagine
35 and the liberated ammonia was determined with an Ammonia kit from Boehringer Mannheim

(cat. no. 1 112 732) based on glutamate dehydrogenase and NADH oxidation to NAD⁺ (can be measured as a decrease in A₃₇₅). Hence the decrease in absorbance at 375 nm was taken as a measure of asparaginase activity.

Asparagine substrate :	10mg/ml L-asparagine (Sigma A-7094) was dissolved in Universal buffers and pH was adjusted to the indicated pH-values with HCl or NaOH.
Temperature :	controlled
Universal buffers :	100 mM succinic acid, 100 mM HEPES, 100 mM CHES, 100 mM CABS, 1 mM CaCl ₂ , 150 mM KCl, 0.01% Triton X-100 adjusted to pH-values 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0 and 12.0 with HCl or NaOH.
Stop reagent :	500 mM TCA (Trichloroacetic acid).
Assay buffer :	1.0M KH ₂ PO ₄ /NaOH, pH 7.5.
Ammonia reagent A :	1 NADH tablet + 1.0 ml Bottle 1 (contain 2-oxoglutarate (second substrate) and buffer) + 2.0 ml Assay buffer.
Ammonia reagent B :	40 micro-l Bottle 3 (contain glutamate dehydrogenase) + 1460 micro-l Assay buffer.

5 450 micro-l asparagine substrate was placed on ice in an Eppendorf tube. 50 micro-l asparaginase sample (diluted in 0.01% Triton X-100) was added. The assay was initiated by transferring the Eppendorf tube to an Eppendorf thermomixer, which was set to the assay temperature. The tube was incubated for 15 minutes on the Eppendorf thermomixer at its highest shaking rate (1400 rpm). The incubation was stopped by transferring the tube back to
10 the ice bath and adding 500 micro-l Stop reagent. The tube was vortexed and centrifuged shortly in an icecold centrifuge to precipitate the proteins in the tube. The amount of ammonia liberated by the enzyme was measured by the following procedure: 20 micro-l supernatant was transferred to a microtiter plate, 200 micro-l Ammonia reagent A was added and A₃₇₅ was read (A₃₇₅(initial)). Then 50 micro-l Ammonia reagent B was added and after 10 minutes at room
15 temperature the plate was read again (A₃₇₅(final)). A₃₇₅(initial) – A₃₇₅(final) was a measure of asparaginase activity. A buffer blind was included in the assay (instead of enzyme) and the decrease in A₃₇₅ in the buffer blind was subtracted from the enzyme samples.

pH-activity, pH-stability, and temperature-activity of asparaginase

20 The above asparaginase assay was used for obtaining the pH-activity profile, the pH-stability profile as well as the temperature-activity profile at pH 7.0. For the pH-stability profile the asparaginase was diluted 7x in the Universal buffers and incubated for 2 hours at 37°C.

After incubation the asparaginase samples were transferred to neutral pH, before assay for residual activity, by dilution in the pH 7 Universal buffer.

The results for the: pH-activity profile at 37°C were as follows, relative to the residual activity at after 2 hours at pH 7.0 and 5°C :

pH	Asparaginase
2	0.00
3	0.01
4	0.10
5	0.53
6	0.95
7	1.00
8	0.66
9	0.22
10	0.08
11	0.00

5

The results for the pH-stability profile (residual activity after 2 hours at 37°C) were as follows:

pH	Asparaginase
2.0	0.00
3.0	0.00
4.0	1.06
5.0	1.08
6.0	1.09
7.0	1.09
8.0	0.92
9.0	0.00
10.0	0.00
11.0	0.00
12.0	0.00
	1.00

The results for the temperature activity profile (at pH 7.0) were as follows:

Temp (°C)	Asparaginase
15	0.24
25	0.39
37	0.60
50	0.81
60	1.00
70	0.18

Other characteristics

The relative molecular weight as determined by SDS-PAGE was seen as a broad band (a smear) at M_r = 40-65 kDa.

N-terminal sequencing showed four different terminals, corresponding to residues 27-37, 30-40, 75-85 and 80-91 of SEQ ID NO: 2, respectively.

Example 3: Cloning of asparaginase from *Penicillium citrinum*

Penicillium citrinum was grown in MEX-1 medium (Medium B in WO 98/38288) in flasks shaken at 150RPM at 26°C for 3 and 4 days. Mycelium was harvested, a cDNA library constructed, and cDNAs encoding secreted peptides were selected and sequenced by the methods described in WO 03/044049. Comparison to known sequences by methods described in WO 03/044049 indicated that *Penicillium* sequence ZY132299 encoded an asparaginase. The complete sequence of the corresponding cDNA was determined and is presented as SEQ ID NO: 11, and the peptide translated from this sequence is presented as SEQ ID NO: 12.

15 Example 4: Effect of asparaginase on acrylamide content in potato chips

Asparaginase from *A. oryzae* having the amino acid sequence shown in SEQ ID NO: 2 was prepared and purified as in Examples 1-2 and added at various dosages to potato chips made from 40 g of water, 52.2 g of dehydrated potato flakes, 5.8 g of potato starch and 2 g of salt.

20 The flour and dry ingredients were mixed for 30 sec. The salt and enzyme were dissolved in the water, and the solution was adjusted to 30°C. The solution was added to the flour. The dough was further mixed for 15 min. The mixed dough was placed in a closed plastic bag and allowed to rest for 15 min at room temperature.

The dough was then initially compressed for 60 sec in a dough press.

25 The dough was sheeted and folded in a noodle roller machine until an approx. 5-10 mm dough is obtained. The dough was then rolled around a rolling pin and allowed to rest for

30 min in a plastic bag at room temperature. The dough was sheeted further to a final sheet thickness of approx 1.2 mm.

The sheet was cut into squares of approx 3 x 5 cm.

The sheets were placed in a frying basket, placed in an oil bath and fried for 45 sec at 180° C. The noodle basket was held at a 45° angle until the oil stopped dripping. The products were removed from the basket and left to cool on dry absorbent paper.

The potato chips were homogenized and analyzed for acrylamide. The results were as follows:

Asparaginase dosage U/kg potato dry matter	Acrylamide Micro-g per kg
0	5,200
100	4,600
500	3,100
1000	1,200
2000	150

The results demonstrate that the asparaginase treatment is effective to reduce the acrylamide content in potato chips, that the acrylamide reduction is clearly dosage dependent, and that the acrylamide content can be reduced to a very low level.

Example 5: Effect of various enzymes on acrylamide content in potato chips

Potato chips were made as follows with addition of enzyme systems which are capable of reacting on asparagine, as indicated below.

Recipe:

Tap water	40 g
Potato flakes dehydrated	52.2 g
Potato starch	5.8 g
Salt	2 g

Dough Procedure:

The potato flakes and potato starch are mixed for 30 sec in a mixer at speed 5. Salt and enzyme are dissolved in the water. The solution is adjusted to 30°C +/- 1°C. Stop mixer, add all of the salt/enzyme solution to flour. The dough is further mixed for 15 min.

Place mixed dough in plastic bag, close bag and allow the dough to rest for 15 min at room temperature.

The dough is then initially compressed for 60 sec in a dough press.

The dough is sheeted and folded in a noodle roller machine until an approx. 5-10 mm dough is obtained. The dough is then rolled around a rolling pin and the dough is allowed to rest for 30 min in a plastic bag at room temperature. The dough is sheeted further to a final sheet thickness of approx 1.2 mm.

- 5 Cut the sheet into squares of approx 3 x 5 cm.

Sheets are placed in a frying basket, placed in the oil bath and fried for 60 sec at 180°C. Hold the noodle basket at a 45° angle and let the product drain until oil stops dripping. Remove the products from the basket and leave them to cool on dry absorbent paper.

The results from acrylamide analysis were as follows:

Enzyme	Enzyme dosage per kg of potato dry matter	Acrylamide Micro-g per kg
None (control)	0	4,100
Asparaginase from <i>Erwinia Chrysanthemi</i> A-2925	1000 U/kg	150
Glutaminase (product of Daiwa)	50 mg enzyme protein/kg	1,800
Amino acid oxidase from <i>Trichoderma harzianum</i> described in WO 9425574.	50 mg enzyme protein/kg	1,300
Laccase from <i>Myceliophthora thermophila</i> + peroxidase from <i>Coprinus</i>	5000 LAMU/kg + 75 mg enzyme protein/kg	2,000

10

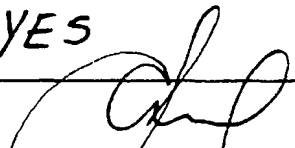
The results demonstrate that all the tested enzyme systems are effective in reducing the acrylamide content of potato chips.

PCT

Original (for SUBMISSION) - printed on 10.10.2003 09:39:26 AM

0-1	Form - PCT/RO/134 (EASY) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)	
0-1-1	Prepared using	PCT-EASY Version 2.92 (updated 01.07.2003)
0-2	International Application No.	
0-3	Applicant's or agent's file reference	10347-WO
1	The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:	
1-1	page	4
1-2	line	5-7
1-3	Identification of Deposit	
1-3-1	Name of depositary institution	DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH
1-3-2	Address of depositary institution	Mascheroder Weg 1b, D-38124 Braunschweig, Germany
1-3-3	Date of deposit	06 October 2003 (06.10.2003)
1-3-4	Accession Number	DSMZ 15960
1-4	Additional Indications	NONE
1-5	Designated States for Which Indications are Made	all designated States
1-6	Separate Furnishing of Indications These indications will be submitted to the International Bureau later	NONE

FOR RECEIVING OFFICE USE ONLY

0-4	This form was received with the international application: (yes or no)	YES
0-4-1	Authorized officer	

FOR INTERNATIONAL BUREAU USE ONLY

0-5	This form was received by the international Bureau on:	
0-5-1	Authorized officer	

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE**DSMZ**
Deutsche Sammlung von
Mikroorganismen und
Zellkulturen GmbH

INTERNATIONAL FORM

Novozymes A/S
Krogshøjvej 36
DK-2880 BagsvaerdVIABILITY STATEMENT
issued pursuant to Rule 10.2 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. DEPOSITOR		II. IDENTIFICATION OF THE MICROORGANISM	
Name: Novozymes A/S Krogshøjvej 36 Address: DK-2880 Bagsvaerd		Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 15960 Date of the deposit or the transfer ¹ : 2003-10-06	
III. VIABILITY STATEMENT			
The viability of the microorganism identified under II above was tested on 2003-10-06 On that date, the said microorganism was (X) ² viable () ² no longer viable			
IV. CONDITIONS UNDER WHICH THE VIABILITY TEST HAS BEEN PERFORMED⁴			
V. INTERNATIONAL DEPOSITARY AUTHORITY			
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig		Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s): V. Weib Date: 2003-10-13	


- ¹ Indicate the date of original deposit or, where a new deposit or a transfer has been made, the most recent relevant date (date of the new deposit or date of the transfer).
² In the cases referred to in Rule 10.2(a) (ii) and (iii), refer to the most recent viability test.
³ Mark with a cross the applicable box.
⁴ Fill in if the information has been requested and if the results of the test were negative.

BUDAPEST TREATY ON THE INTERNATIONAL
RECOGNITION OF THE DEPOSIT OF MICROORGANISMS
FOR THE PURPOSES OF PATENT PROCEDURE

INTERNATIONAL FORM

Novozymes A/S
Krogshøjvej 36
DK-2880 Bagsvaerd

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
issued pursuant to Rule 7.1 by the
INTERNATIONAL DEPOSITARY AUTHORITY
identified at the bottom of this page

I. IDENTIFICATION OF THE MICROORGANISM	
Identification reference given by the DEPOSITOR: NN049697	Accession number given by the INTERNATIONAL DEPOSITARY AUTHORITY: DSM 15960
II. SCIENTIFIC DESCRIPTION AND/OR PROPOSED TAXONOMIC DESIGNATION	
The microorganism identified under I. above was accompanied by: () a scientific description (x) a proposed taxonomic designation (Mark with a cross where applicable).	
III. RECEIPT AND ACCEPTANCE	
This International Depositary Authority accepts the microorganism identified under I. above, which was received by it on 2003-10-06 (Date of the original deposit) ¹ .	
IV. RECEIPT OF REQUEST FOR CONVERSION	
The microorganism identified under I above was received by this International Depositary Authority on _____ (date of original deposit) and a request to convert the original deposit to a deposit under the Budapest Treaty was received by it on _____ (date of receipt of request for conversion).	
V. INTERNATIONAL DEPOSITARY AUTHORITY	
Name: DSMZ-DEUTSCHE SAMMLUNG VON MIKROORGANISMEN UND ZELLKULTUREN GmbH Address: Mascheroder Weg 1b D-38124 Braunschweig	Signature(s) of person(s) having the power to represent the International Depositary Authority or of authorized official(s):  Date: 2003-10-13

¹ Where Rule 6.4 (d) applies, such date is the date on which the status of international depositary authority was acquired.

CLAIMS

1. A method of preparing a heat-treated product, comprising the sequential steps of:
 - a) providing a raw material which comprises carbohydrate, protein and water
 - b) treating the raw material with an enzyme capable of reacting on asparagine or glutamine (optionally substituted) as a substrate, a laccase or a peroxidase, and
 - 5 c) heat treating to reach a final water content below 35 % by weight.
2. The method of the preceding claim wherein the enzyme capable of reacting on asparagine or glutamine (optionally substituted) as a substrate is an asparaginase, a glutaminase, an L-amino acid oxidase, a glycosylasparaginase, a glycoamidase (peptide N-glycosidase) or
10 a peptidoglutaminase.
3. The method of the preceding claim wherein the asparaginase has an amino acid sequence which is at least 90 % identical to SEQ ID NO: 2 (optionally truncated to residues 27-378, 30-378, 75-378 or 80-378), 4, 6, 8, 10, 12 or 13.
4. The method of any preceding claim which further comprises treating the raw material
15 with an oxidoreductase capable of reacting with a reducing sugar as a substrate.
5. The method of the preceding claim wherein the oxidoreductase capable of reacting with a reducing sugar as a substrate is a glucose oxidase, a pyranose oxidase, a hexose oxidase, a galactose oxidase (EC 1.1.3.9) or a carbohydrate oxidase which has a higher activity on maltose than on glucose.
- 20 6. The method of any preceding claim wherein the raw material is in the form of a dough and the enzyme treatment comprises mixing the enzyme into the dough and optionally holding.
7. The method of any preceding claim wherein the raw material comprises intact vegetable pieces and the enzyme treatment comprises immersing the potato pieces in an aqueous solution of the enzyme.
- 25 8. The method of any preceding claim wherein the raw material comprises a potato product.

9. A polypeptide having asparaginase activity and having an amino acid sequence which is at least 90 % identical with SEQ ID NO: 2 (optionally truncated to residues 27-378, 30-378, 75-378 or 80-378) or SEQ ID NO: 12.
10. A polynucleotide encoding the polypeptide of the preceding claim.
- 5 11. A polynucleotide which encodes an asparaginase and which comprises a nucleotide sequence which is at least 90 % identical to the coding sequences of SEQ ID NO: 1 or 11.

10347-WO-ST25
SEQUENCE LISTING

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<120> Method of Preparing an Edible Product

<130> 10347-WO

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10347-WO-ST25

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10347-wo-ST25

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10347-WO-ST25

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												Met 1	Thr	Lys	Leu	Ser 5	Phe	Lys		
atc	atc	aca	ctc	gcg	gct	atg	ata	gcc	gtt	ggg	aat	gcc	tct	ccg	ttt					161
Ile	Ile	Thr 10	Leu	Ala	Ala	Met	Ile 15	Ala	Val	Gly	Asn	Ala 20	Ser	Pro	Phe					
gtc	tac	ccc	cga	gca	acc	agc	cca	aac	agt	aca	tat	gtc	ttc	acc	aac					209
Val	Tyr 25	Pro	Arg	Ala	Thr	Ser 30	Pro	Asn	Ser	Thr	Tyr 35	Val	Phe	Thr	Asn					
tcg	cat	ggc	ttg	aac	ttc	acc	cag	atg	aac	acg	acg	ctc	cct	aat	gtc					257
Ser 40	His	Gly	Leu	Asn	Phe 45	Thr	Gln	Met	Asn	Thr 50	Thr	Leu	Pro	Asn	Val 55					
acc	atc	ctc	gca	acc	ggc	ggt	acc	att	gcc	ggc	tcc	agc	aac	gac	aac					305
Thr	Ile	Leu	Ala	Thr 60	Gly	Gly	Thr	Ile	Ala 65	Gly	Ser	Ser	Asn	Asp 70	Asn					
acc	gcc	aca	aca	ggc	tac	acg	gcc	ggc	gcg	atc	ggc	atc	cag	cag	ctc					353
Thr	Ala	Thr	Thr 75	Gly	Tyr	Thr	Ala	Gly 80	Ala	Ile	Gly	Ile	Gln 85	Gln	Leu					
atg	gat	gcc	gtc	cct	gag	atg	cta	gac	gtt	gct	aac	gtg	gcc	ggc	atc					401
Met	Asp	Ala 90	Val	Pro	Glu	Met	Leu 95	Asp	Val	Ala	Asn	Val 100	Ala	Gly	Ile					
cag	gtc	gcc	aat	gtc	ggc	agc	ccc	gac	gtg	acg	tct	tcc	ctt	ctg	ctc					449
Gln	Val 105	Ala	Asn	Val	Gly	Ser 110	Pro	Asp	Val	Thr	Ser 115	Ser	Leu	Leu	Leu					
cac	atg	gcc	agg	acc	atc	aac	gag	gtc	gtc	tgc	gac	gac	ccc	acc	atg					497
His 120	Met	Ala	Arg	Thr	Ile 125	Asn	Glu	Val	Val	Cys 130	Asp	Asp	Pro	Thr	Met 135					
agc	ggc	gcc	gtc	atc	acg	cac	ggc	acc	gac	acg	ctc	gag	gag	acg	gcc					545
Ser	Gly	Ala	Val	Ile 140	Thr	His	Gly	Thr	Asp 145	Thr	Leu	Glu	Glu	Thr 150	Ala					
ttc	ttc	ctc	gac	gct	aca	gtc	aac	tgc	ggc	aag	ccc	atc	gtc	gtc	gtc					593
Phe	Phe	Leu	Asp 155	Ala	Thr	Val	Asn	Cys 160	Gly	Lys	Pro	Ile	Val 165	Val	Val					
ggc	gcc	atg	cgg	ccc	gca	acc	gcc	atc	tcc	gcc	gac	ggc	ccg	ttc	aac					641
Gly	Ala	Met 170	Arg	Pro	Ala	Thr	Ala 175	Ile	Ser	Ala	Asp	Gly 180	Pro	Phe	Asn					
ctc	ctc	cag	gcc	gtg	acc	gtc	gcc	gcg	cac	ccc	act	gcg	cg	aac	cgt					689
Leu	Leu 185	Gln	Ala	Val	Thr	Val 190	Ala	Ala	His	Pro	Thr 195	Ala	Arg	Asn	Arg					
ggt	gcg	ctg	gtc	gtc	atg	aac	gac	cg	att	gtg	tcc	gcg	tac	tac	gtc					737
Gly 200	Ala	Leu	Val	Val	Met 205	Asn	Asp	Arg	Ile	Val 210	Ser	Ala	Tyr	Tyr	Val 215					
tcc	aag	aca	aac	gcc	aac	acc	atg	gac	acc	ttc	aag	gcc	gtc	gag	atg					785
Ser	Lys	Thr	Asn	Ala 220	Asn	Thr	Met	Asp	Thr 225	Phe	Lys	Ala	Val	Glu 230	Met					

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tcc atc ccc aga gtc gac atc ctc tac tcg tac cag gat atg caa aac 929
 Ser Ile Pro Arg Val Asp Ile Leu Tyr Ser Tyr Gln Asp Met Gln Asn
 265 270 275
 gat acg ctc tac gac gcc gtc gac aac ggc gcg aaa ggc atc gtc gta a 978
 Asp Thr Leu Tyr Asp Ala Val Asp Asn Gly Ala Lys Gly Ile Val Val
 280 285 290 295
 gtccagcccc ttcttaaagc cctcaccgga tcaaccgctg aaattgaacc taatccagat 1038
 cgccggctcc ggcgcag ga agc gtc tca agt ggc tac tac gat gcc atc 1087
 Arg Ser Val Ser Ser Gly Tyr Tyr Asp Ala Ile
 300 305
 gac gac atc gca tcc acg cac tcc ctc cct gtc gtc ctc agc act cgc 1135
 Asp Asp Ile Ala Ser Thr His Ser Leu Pro Val Val Leu Ser Thr Arg
 310 315 320
 acc ggc aac ggc gaa gtc gcc atc aca gac agc gag acc aca att gag 1183
 Thr Gly Asn Gly Glu Val Ala Ile Thr Asp Ser Glu Thr Thr Ile Glu
 325 330 335
 agc ggc ttc ctg aac ccg cag aaa ggc cgc atc ctg ctc ggt ctg ctg 1231
 Ser Gly Phe Leu Asn Pro Gln Lys Ala Arg Ile Leu Leu Gly Leu Leu
 340 345 350
 ctt gct gag gat aag gga ttc aag gag atc aaa gag gcg ttc gcg aag 1279
 Leu Ala Glu Asp Lys Gly Phe Lys Glu Ile Lys Glu Ala Phe Ala Lys
 355 360 365 370
 aac ggg gtt gct tgattatgtc cttccttggt ttgggtggca tttgtggtt 1330
 Asn Gly Val Ala

<210> 6
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 <212> PRT
 <213> *Aspergillus fumigatus*

<400> 6

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Val Gly Asn Ala Ser Pro Phe Val Tyr Pro Arg Ala Thr Ser Pro Asn
20 25 30

Ser Thr Tyr Val Phe Thr Asn Ser His Gly Leu Asn Phe Thr Gln Met
35 40 45

Asn Thr Thr Leu Pro Asn Val Thr Ile Leu Ala Thr Gly Gly Thr Ile
50 55 60

Ala Gly Ser Ser Asn Asp Asn Thr Ala Thr Thr Gly Tyr Thr Ala Gly
65 70 75 80

Ala Ile Gly Ile Gln Gln Leu Met Asp Ala Val Pro Glu Met Leu Asp
85 90 95

Val Ala Asn Val Ala Gly Ile Gln Val Ala Asn Val Gly Ser Pro Asp
Page 9

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 <211> 1260
 <212> DNA
 <213> *Fusarium graminearum*

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 <222> (105)..(1217)

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 tctccagtct ctccaagtgt tgtgccctct tgtgttagcc cagc atg tcg ccc tct 116
 Met Ser Pro Ser
 1
 ttc cac tcc cta ctc gct atc gca acc ctt gca ggc tca gct gcc ctt 164
 Phe His Ser Leu Leu Ala Ile Ala Thr Leu Ala Gly Ser Ala Ala Leu
 5 10 15 20
 gca tcc ccg atc ccg gag cca gaa aca ccg cag ctt atc ccc cgg gct 212
 Ala Ser Pro Ile Pro Glu Pro Glu Thr Pro Gln Leu Ile Pro Arg Ala
 25 30 35
 gtt ggt gac ttt gag tgc ttc aac gct agt ctt ccc aac atc acc atc 260
 Val Gly Asp Phe Glu Cys Phe Asn Ala Ser Leu Pro Asn Ile Thr Ile
 40 45 50
 ttc gcg act ggt ggt acc atc gct ggt tct gct ggt tct gcc gat cag 308
 Phe Ala Thr Gly Gly Thr Ile Ala Gly Ser Ala Gly Ser Ala Asp Gln
 55 60 65
 act acg ggt tac cag gct ggt gca ttg ggt atc caa gcg ttg atc gac 356
 Thr Thr Gly Tyr Gln Ala Gly Ala Leu Gly Ile Gln Ala Leu Ile Asp
 70 75 80
 gct gtc ccg caa ctc tgc aac gtc tcc aac gtc agg ggt gtg cag atc 404
 Ala Val Pro Gln Leu Cys Asn Val Ser Asn Val Arg Gly Val Gln Ile
 85 90 95 100
 gcc aac gtt gat agc ggc gat gta aac tct act atc ctg acc act ttg 452
 Ala Asn Val Asp Ser Gly Asp Val Asn Ser Thr Ile Leu Thr Thr Leu
 105 110 115
 gcg cat cgc atc cag act gat ctt gac aac cct cac atc caa ggt gtt 500
 Ala His Arg Ile Gln Thr Asp Leu Asp Asn Pro His Ile Gln Gly Val
 120 125 130
 gtc gtc acc cat ggc aca gac act ctc gag gag tct tca ttt ttc ctc 548
 Val Val Thr His Gly Thr Asp Thr Leu Glu Glu Ser Ser Phe Phe Leu
 135 140 145
 gat ctc act gtc caa agt gaa aag cct gtt gtt atg gtt gga tcc atg 596
 Asp Leu Thr Val Gln Ser Glu Lys Pro Val Val Met Val Gly Ser Met
 150 155 160
 cgt cct gcc act gcc atc agc gct gat ggt ccc atc aac ctc ctg tct 644
 Arg Pro Ala Thr Ala Ile Ser Ala Asp Gly Pro Ile Asn Leu Leu Ser
 165 170 175 180
 gct gtt cga ttg gca ggt agc aag agt gcc aag ggt cgc ggt aca atg 692
 Ala Val Arg Leu Ala Gly Ser Lys Ser Ala Lys Gly Arg Gly Thr Met
 185 190 195

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Ile Val Leu Asn Asp Lys Ile Ala Ser Ala Arg Tyr Thr Val Lys Ser	
200 205 210	
cac gcc aat gct gtc cag act ttc att gcc gaa gat caa ggt tat ctt	788
His Ala Asn Ala Val Gln Thr Phe Ile Ala Glu Asp Gln Gly Tyr Leu	
215 220 225	
ggt gcc ttt gaa aac att cag ccc gtc ttc tgg tac cct gct agt cga	836
Gly Ala Phe Glu Asn Ile Gln Pro Val Phe Trp Tyr Pro Ala Ser Arg	
230 235 240	
cca cta ggt cac cac tat ttc aac att agt gct agc tca cct aag aag	884
Pro Leu Gly His His Tyr Phe Asn Ile Ser Ala Ser Ser Pro Lys Lys	
245 250 255 260	
gct ctt cct cag gtt gac gtt ttg tac ggc cac caa gaa gcg gac ccc	932
Ala Leu Pro Gln Val Asp Val Leu Tyr Gly His Gln Glu Ala Asp Pro	
265 270 275	
gag ctt ttc caa gct gct gtc gat agc ggc gcc cag ggc att gtt ctc	980
Glu Leu Phe Gln Ala Ala Val Asp Ser Gly Ala Gln Gly Ile Val Leu	
280 285 290	
gct ggt ctt ggc gct gga ggc tgg cct gac gaa gct gct gat gag atc	1028
Ala Gly Leu Gly Ala Gly Gly Trp Pro Asp Glu Ala Ala Asp Glu Ile	
295 300 305	
aag aag gtc ttg aac gag act aac att cct gtt gtt gtc agc cgt cgt	1076
Lys Lys Val Leu Asn Glu Thr Asn Ile Pro Val Val Val Ser Arg Arg	
310 315 320	
act gct tgg ggt tac gtt gga gag agg cct ttc ggt atc ggt gct ggg	1124
Thr Ala Trp Gly Tyr Val Gly Glu Arg Pro Phe Gly Ile Gly Ala Gly	
325 330 335 340	
tac ttg aac cct tcc aag gcc aga atc caa ctg caa ctt gcg ctt gag	1172
Tyr Leu Asn Pro Ser Lys Ala Arg Ile Gln Leu Gln Leu Ala Leu Glu	
345 350 355	
aag aag ctt tct gtg gag gag atc caa gac ata ttc gag tat gtt	1217
Lys Lys Leu Ser Val Glu Glu Ile Gln Asp Ile Phe Glu Tyr Val	
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 <212> PRT
 <213> Fusarium graminearum

<400> 8

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 20 25 30

Ile Pro Arg Ala Val Gly Asp Phe Glu Cys Phe Asn Ala Ser Leu Pro
 35 40 45

Asn Ile Thr Ile Phe Ala Thr Gly Gly Thr Ile Ala Gly Ser Ala Gly
 50 55 60

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Ser Ala Asp Gln Thr Thr Gly Tyr Gln Ala Gly Ala Leu Gly Ile Gln
 65 70 75 80
 Ala Leu Ile Asp Ala Val Pro Gln Leu Cys Asn Val Ser Asn Val Arg
 85 90 95
 Gly Val Gln Ile Ala Asn Val Asp Ser Gly Asp Val Asn Ser Thr Ile
 100 105 110
 Leu Thr Thr Leu Ala His Arg Ile Gln Thr Asp Leu Asp Asn Pro His
 115 120 125
 Ile Gln Gly Val Val Val Thr His Gly Thr Asp Thr Leu Glu Glu Ser
 130 135 140
 Ser Phe Phe Leu Asp Leu Thr Val Gln Ser Glu Lys Pro Val Val Met
 145 150 155 160
 Val Gly Ser Met Arg Pro Ala Thr Ala Ile Ser Ala Asp Gly Pro Ile
 165 170 175
 Asn Leu Leu Ser Ala Val Arg Leu Ala Gly Ser Lys Ser Ala Lys Gly
 180 185 190
 Arg Gly Thr Met Ile Val Leu Asn Asp Lys Ile Ala Ser Ala Arg Tyr
 195 200 205
 Thr Val Lys Ser His Ala Asn Ala Val Gln Thr Phe Ile Ala Glu Asp
 210 215 220
 Gln Gly Tyr Leu Gly Ala Phe Glu Asn Ile Gln Pro Val Phe Trp Tyr
 225 230 235 240
 Pro Ala Ser Arg Pro Leu Gly His His Tyr Phe Asn Ile Ser Ala Ser
 245 250 255
 Ser Pro Lys Lys Ala Leu Pro Gln Val Asp Val Leu Tyr Gly His Gln
 260 265 270
 Glu Ala Asp Pro Glu Leu Phe Gln Ala Ala Val Asp Ser Gly Ala Gln
 275 280 285
 Gly Ile Val Leu Ala Gly Leu Gly Ala Gly Gly Trp Pro Asp Glu Ala
 290 295 300
 Ala Asp Glu Ile Lys Lys Val Leu Asn Glu Thr Asn Ile Pro Val Val
 305 310 315 320
 Val Ser Arg Arg Thr Ala Trp Gly Tyr Val Gly Glu Arg Pro Phe Gly
 325 330 335

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Ile Gly Ala Gly Tyr Leu Asn Pro Ser Lys Ala Arg Ile Gln Leu Gln
 340 345 350

Leu Ala Leu Glu Lys Lys Leu Ser Val Glu Glu Ile Gln Asp Ile Phe
 355 360 365

Glu Tyr Val
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 Met Met Pro Ser Val Arg Arg Phe His Gly Gln Thr
 1 5 10
 atg gtc gcc gcc gct cct tct att tgc tca ggg cct gca gca tcg tcc 160
 Met Val Ala Ala Ala Pro Ser Ile Cys Ser Gly Pro Ala Ala Ser Ser
 15 20 25
 acc atc aag atg gct tca tcg tca gct tcg tgg acg act tat ctg tgg 208
 Thr Ile Lys Met Ala Ser Ser Ala Ser Trp Thr Thr Tyr Leu Trp
 30 35 40
 cgg ctt atc cta gct gtg ctg gct cct tca acg gcc ctg ctg cct ttt 256
 Arg Leu Ile Leu Ala Val Leu Ala Pro Ser Thr Thr Ala Leu Leu Pro Phe
 45 50 55 60
 ggt gcg tgg gtt gtt tcg gtc tgg gga tct cct gtc ctc gac cta cac 304
 Gly Ala Trp Val Ser Val Trp Gly Ser Pro Val Leu Asp Leu His
 65 70 75
 gtc caa cct cac ttc tcg gtt caa caa aaa gcg cca ata cag acg ggc 352
 Val Gln Pro His Phe Ser Val Gln Gln Lys Ala Pro Ile Gln Thr Gly
 80 85 90
 atc cct ttc gaa att tcg acc acc tca gga ttc aac tgc ttc aat ccc 400
 Ile Pro Phe Glu Ile Ser Thr Thr Ser Gly Phe Asn Cys Phe Asn Pro
 95 100 105
 aat ctt ccc aac gtc act att tat gcc acc gga ggt act att gct ggc 448
 Asn Leu Pro Asn Val Thr Ile Tyr Ala Thr Gly Gly Thr Ile Ala Gly
 110 115 120
 tcc gca agc tcg gct gat cag acc acg gga tac cgg tca gct gcg tta 496
 Ser Ala Ser Ser Ala Asp Gln Thr Thr Gly Tyr Arg Ser Ala Ala Leu
 125 130 135 140
 gga gtt gat tct ctc att gat gca gta ccc caa ttg tgc aat gta gcc 544
 Gly Val Asp Ser Leu Ile Asp Ala Val Pro Gln Leu Cys Asn Val Ala
 145 150 155
 aat gtg aga ggt gtc cag ttt gcc aac acg gac agc ata gac atg agc 592

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agt Ser	ccg Pro 190	ttt Phe	act Thr	caa Gln	ggc Gly	gca Ala 195	gtt Val	gtg Val	acg Thr	cac His	gga Gly 200	act Thr	gat Asp	act Thr	ctg Leu	688
gat Asp 205	gaa Glu	tct Ser	gcc Ala	ttc Phe	ttt Phe 210	ctg Leu	gat Asp	ctt Leu	act Thr	atc Ile 215	cag Gln	agc Ser	gac Asp	aag Lys	ccc Pro 220	736
gtg Val	gtc Val	gtg Val	aca Thr	ggc Gly 225	tca Ser	atg Met	cgc Arg	ccg Pro	gca Ala 230	act Thr	gct Ala	atc Ile	agc Ser	gca Ala 235	gat Asp	784
gga Gly	cca Pro	atg Met	aat Asn 240	ctt Leu	ttg Leu	tca Ser	tcg Ser	gtg Val 245	aca Thr	ttg Leu	gca Ala	gca Ala	gca Ala 250	gcg Ala	agt Ser	832
gct Ala	cga Arg	ggc Gly 255	aga Arg	gga Gly	gtg Val	atg Met	att Ile 260	gcc Ala	atg Met	aat Asn	gat Asp	cgc Arg 265	att Ile	gga Gly	tct Ser	880
gct Ala	cgt Arg 270	ttt Phe	acg Thr	acc Thr	aaa Lys	gtc Val 275	aac Asn	gcc Ala	aac Asn	cat His	ttg Leu 280	gac Asp	gcc Ala	ttc Phe	caa Gln	928
gcc Ala 285	cct Pro	gac Asp	agt Ser	ggc Gly	atg Met 290	ctg Leu	gga Gly	aca Thr	ttc Phe	gtc Val 295	aac Asn	gtt Val	cag Gln	cca Pro	gtg Val 300	976
ttt Phe	ttc Phe	tat Tyr	ccg Pro	cca Pro 305	tca Ser	cga Arg	cct Pro	ctt Leu	ggc Gly 310	cac His	cgt Arg	cat His	ttt Phe	gat Asp 315	ctg Leu	1024
cgg Arg	ccc Pro	atc Ile	acc Thr 320	aac Asn	aac Asn	ggc Gly	cgc Arg	cgg Arg 325	ttc Phe	gga Gly	cgc Arg	tct Ser	aca Thr 330	gcc Ala	ccc Pro	1072
gga Gly	gca Ala	gga Gly 335	tca Ser	tca Ser	gca Ala	cta Leu	ccc Pro 340	cag Gln	gtg Val	gac Asp	gtg Val	ctc Leu 345	tac Tyr	gct Ala	tac Tyr	1120
cag Gln	gag Glu 350	ctc Leu	agc Ser	gtg Val	ggc Gly	atg Met 355	ttc Phe	cag Gln	gcg Ala	gcc Ala	atc Ile 360	gac Asp	ctt Leu	gga Gly	gcg Ala	1168
cag Gln 365	ggc Gly	atc Ile	gtt Val	cta Leu	gcg Ala 370	gga Gly	atg Met	ggc Gly	gct Ala	gga Gly 375	ttc Phe	tgg Trp	acg Thr	tcc Ser	aaa Lys 380	1216
ggt Gly	acc Thr	gag Glu	gag Glu	att Ile 385	cgg Arg	cgt Arg	atc Ile	gtc Val	cac His 390	gag Glu	acc Thr	gat Asp	att Ile	ccc Pro 395	gtg Val	1264
ata Ile	gtg Val	agc Ser	cga Arg 400	aga Arg	ccg Pro	gaa Glu	ggc Gly	ggc Gly 405	ttc Phe	gtc Val	gga Gly	cca Pro	tgt Cys 410	gag Glu	gca Ala	1312
gga Gly	atc Ile	ggc Gly 415	gcg Ala	ggc Gly	ttt Phe	ttg Leu	aat Asn 420	ccg Pro	caa Gln	aag Lys	gcg Ala	agg Arg 425	atc Ile	cag Gln	ctc Leu	1360
caa	ctg	gcc	ctg	gag	acc	aag	atg	gac	aat	gat	gcc	atc	aaa	gcc	ctg	1408

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Gln Leu Ala Leu Glu Thr Lys Met Asp Asn Asp Ala Ile Lys Ala Leu
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 Phe Glu His Ser Gly Val His
 445 450

agcaacacca c 1470

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 <211> 451
 <212> PRT
 <213> Fusarium graminearum

<400> 10

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Ala Pro Ser Ile Cys Ser Gly Pro Ala Ala Ser Ser Thr Ile Lys Met
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Ala Ser Ser Ser Ala Ser Trp Thr Thr Tyr Leu Trp Arg Leu Ile Leu
 35 40 45

Ala Val Leu Ala Pro Ser Thr Ala Leu Leu Pro Phe Gly Ala Trp Val
 50 55 60

Val Ser Val Trp Gly Ser Pro Val Leu Asp Leu His Val Gln Pro His
 65 70 75 80

Phe Ser Val Gln Gln Lys Ala Pro Ile Gln Thr Gly Ile Pro Phe Glu
 85 90 95

Ile Ser Thr Thr Ser Gly Phe Asn Cys Phe Asn Pro Asn Leu Pro Asn
 100 105 110

Val Thr Ile Tyr Ala Thr Gly Gly Thr Ile Ala Gly Ser Ala Ser Ser
 115 120 125

Ala Asp Gln Thr Thr Gly Tyr Arg Ser Ala Ala Leu Gly Val Asp Ser
 130 135 140

Leu Ile Asp Ala Val Pro Gln Leu Cys Asn Val Ala Asn Val Arg Gly
 145 150 155 160

Val Gln Phe Ala Asn Thr Asp Ser Ile Asp Met Ser Ser Ala Met Leu
 165 170 175

Arg Thr Leu Ala Lys Gln Ile Gln Asn Asp Leu Asp Ser Pro Phe Thr
 180 185 190

Gln Gly Ala Val Val Thr His Gly Thr Asp Thr Leu Asp Glu Ser Ala
 195 200 205

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Phe Phe Leu Asp Leu Thr Ile Gln Ser Asp Lys Pro Val Val Val Thr
 210 215 220
 Gly Ser Met Arg Pro Ala Thr Ala Ile Ser Ala Asp Gly Pro Met Asn
 225 230 235 240
 Leu Leu Ser Ser Val Thr Leu Ala Ala Ala Ala Ser Ala Arg Gly Arg
 245 250 255
 Gly Val Met Ile Ala Met Asn Asp Arg Ile Gly Ser Ala Arg Phe Thr
 260 265 270
 Thr Lys Val Asn Ala Asn His Leu Asp Ala Phe Gln Ala Pro Asp Ser
 275 280 285
 Gly Met Leu Gly Thr Phe Val Asn Val Gln Pro Val Phe Phe Tyr Pro
 290 295 300
 Pro Ser Arg Pro Leu Gly His Arg His Phe Asp Leu Arg Pro Ile Thr
 305 310 315 320
 Asn Asn Gly Arg Arg Phe Gly Arg Ser Thr Ala Pro Gly Ala Gly Ser
 325 330 335
 Ser Ala Leu Pro Gln Val Asp Val Leu Tyr Ala Tyr Gln Glu Leu Ser
 340 345 350
 Val Gly Met Phe Gln Ala Ala Ile Asp Leu Gly Ala Gln Gly Ile Val
 355 360 365
 Leu Ala Gly Met Gly Ala Gly Phe Trp Thr Ser Lys Gly Thr Glu Glu
 370 375 380
 Ile Arg Arg Ile Val His Glu Thr Asp Ile Pro Val Ile Val Ser Arg
 385 390 395 400
 Arg Pro Glu Gly Gly Phe Val Gly Pro Cys Glu Ala Gly Ile Gly Ala
 405 410 415
 Gly Phe Leu Asn Pro Gln Lys Ala Arg Ile Gln Leu Gln Leu Ala Leu
 420 425 430
 Glu Thr Lys Met Asp Asn Asp Ala Ile Lys Ala Leu Phe Glu His Ser
 435 440 445
 Gly Val His
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 <211> 1236
 <212> DNA

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<213> Penicillium citrinum

<220>

<221> CDS

<222> (16)..(1152)

<400> 11

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				1				5					10			
cta	gct	gct	acg	agt	tat	gcc	tct	ccc	atc	att	cat	tcc	cgg	gcc	tcc	99
Leu	Ala	Ala	Thr	Ser	Tyr	Ala	Ser	Pro	Ile	Ile	His	Ser	Arg	Ala	Ser	
		15					20					25				
aac	acg	tcc	tat	acc	aac	tct	aat	ggg	ctg	aaa	ttt	aac	cat	ttc	gac	147
Asn	Thr	Ser	Tyr	Thr	Asn	Ser	Asn	Gly	Leu	Lys	Phe	Asn	His	Phe	Asp	
	30					35					40					
gct	tct	ctt	cca	aat	gtg	act	ttg	ctg	gca	act	ggt	gga	act	att	gcc	195
Ala	Ser	Leu	Pro	Asn	Val	Thr	Leu	Leu	Ala	Thr	Gly	Gly	Thr	Ile	Ala	
45					50				55						60	
ggt	aca	agc	gat	gac	aag	act	gct	acg	gca	gga	tat	gaa	tcc	ggg	gct	243
Gly	Thr	Ser	Asp	Asp	Lys	Thr	Ala	Thr	Ala	Gly	Tyr	Glu	Ser	Gly	Ala	
				65				70						75		
tta	ggg	ata	aat	aag	att	ctt	tcc	ggc	atc	cca	gaa	gtt	tat	gac	att	291
Leu	Gly	Ile	Asn	Lys	Ile	Leu	Ser	Gly	Ile	Pro	Glu	Val	Tyr	Asp	Ile	
			80					85					90			
gcc	aac	gtc	aat	gcg	gta	cag	ttt	gac	aat	gtc	aac	agc	ggc	gat	gtc	339
Ala	Asn	Val	Asn	Ala	Val	Gln	Phe	Asp	Asn	Val	Asn	Ser	Gly	Asp	Val	
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tct	yca	tct	ctc	tta	ctg	aac	atg	aca	cat	acc	ctt	caa	aag	acc	gtt	387
Ser	Xaa	Ser	Leu	Leu	Leu	Asn	Met	Thr	His	Thr	Leu	Gln	Lys	Thr	Val	
	110					115					120					
tgt	gat	gac	cct	acg	ata	tct	ggc	gcc	gtc	atc	acc	cat	ggc	acc	gat	435
Cys	Asp	Asp	Pro	Thr	Ile	Ser	Gly	Ala	Val	Ile	Thr	His	Gly	Thr	Asp	
125					130				135						140	
acc	ctg	gaa	gaa	tct	gcc	ttc	ttc	atc	gat	gca	aca	gtc	aac	tgc	ggc	483
Thr	Leu	Glu	Glu	Ser	Ala	Phe	Phe	Ile	Asp	Ala	Thr	Val	Asn	Cys	Gly	
				145				150						155		
aag	ccg	att	gtg	ttc	gtt	ggc	tca	atg	cga	cct	tcc	acc	gca	atc	tct	531
Lys	Pro	Ile	Val	Phe	Val	Gly	Ser	Met	Arg	Pro	Ser	Thr	Ala	Ile	Ser	
			160					165					170			
gcc	gat	ggc	cct	atg	aat	ttg	ctc	cag	gga	gtg	act	gtg	gcc	gct	gac	579
Ala	Asp	Gly	Pro	Met	Asn	Leu	Leu	Gln	Gly	Val	Thr	Val	Ala	Ala	Asp	
		175					180					185				
aaa	cag	gct	aag	aac	cgc	gga	gca	cta	gtc	gtg	ctg	aat	gac	cgc	att	627
Lys	Gln	Ala	Lys	Asn	Arg	Gly	Ala	Leu	Val	Val	Leu	Asn	Asp	Arg	Ile	
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gtc	tct	gct	ttc	ttc	gct	aca	aag	aca	aat	gcg	aat	aca	atg	gac	act	675
Val	Ser	Ala	Phe	Phe	Ala	Thr	Lys	Thr	Asn	Ala	Asn	Thr	Met	Asp	Thr	
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ttc	aag	gct	tat	gaa	caa	ggc	agt	ctt	ggc	atg	att	gtt	tca	aac	aag	723
Phe	Lys	Ala	Tyr	Glu	Gln	Gly	Ser	Leu	Gly	Met	Ile	Val	Ser	Asn	Lys	
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cat ctt gac gac gtg gat gcg atc ccc cgt gtg gat att ctc tac gct      819
His Leu Asp Asp Val Asp Ala Ile Pro Arg Val Asp Ile Leu Tyr Ala
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tac gag gac atg cat agc gac tcc ctt cac agt gct atc aaa aat gga      867
Tyr Glu Asp Met His Ser Asp Ser Leu His Ser Ala Ile Lys Asn Gly
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gcc aag ggc atc gtg gtc gcc ggc gag ggc gca ggt ggt atc tcc acg      915
Ala Lys Gly Ile Val Val Ala Gly Glu Gly Ala Gly Gly Ile Ser Thr
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Asp Phe Ser Asp Thr Thr Ile Asp Glu Ile Ala Ser Lys His Gln Ile Pro
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att atc ctg agc cac aga acc gtg aac gga gaa gtt cct act gct gat     1011
Ile Ile Leu Ser His Arg Thr Val Asn Gly Glu Val Pro Thr Ala Asp
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Ile Thr Gly Asp Ser Ala Lys Thr Arg Ile Ala Ser Gly Met Tyr Asn
                335                340                345

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Pro Gln Gln Ala Arg Val Leu Leu Gly Leu Leu Leu Ala Glu Gly Lys
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aag ttt gag gat att cga act atc ttc gga aaa gct act gtt gcc     1152
Lys Phe Glu Asp Ile Arg Thr Ile Phe Gly Lys Ala Thr Val Ala
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Thr Asn Ser Asn Gly Leu Lys Phe Asn His Phe Asp Ala Ser Leu Pro
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Asn Val Thr Leu Leu Ala Thr Gly Gly Thr Ile Ala Gly Thr Ser Asp
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Asp Lys Thr Ala Thr Ala Gly Tyr Glu Ser Gly Ala Leu Gly Ile Asn
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 Lys Ile Leu Ser Gly Ile Pro Glu Val Tyr Asp Ile Ala Asn Val Asn
 85 90 95
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 Leu Leu Asn Met Thr His Thr Leu Gln Lys Thr Val Cys Asp Asp Pro
 115 120 125
 Thr Ile Ser Gly Ala Val Ile Thr His Gly Thr Asp Thr Leu Glu Glu
 130 135 140
 Ser Ala Phe Phe Ile Asp Ala Thr Val Asn Cys Gly Lys Pro Ile Val
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 Phe Val Gly Ser Met Arg Pro Ser Thr Ala Ile Ser Ala Asp Gly Pro
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 180 185 190
 Asn Arg Gly Ala Leu Val Val Leu Asn Asp Arg Ile Val Ser Ala Phe
 195 200 205
 Phe Ala Thr Lys Thr Asn Ala Asn Thr Met Asp Thr Phe Lys Ala Tyr
 210 215 220
 Glu Gln Gly Ser Leu Gly Met Ile Val Ser Asn Lys Pro Tyr Phe Tyr
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 Tyr Pro Ala Val Glu Pro Asn Ala Lys His Val Val His Leu Asp Asp
 245 250 255
 Val Asp Ala Ile Pro Arg Val Asp Ile Leu Tyr Ala Tyr Glu Asp Met
 260 265 270
 His ser Asp Ser Leu His Ser Ala Ile Lys Asn Gly Ala Lys Gly Ile
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 Thr Ile Asp Glu Ile Ala Ser Lys His Gln Ile Pro Ile Ile Leu Ser
 305 310 315 320
 His Arg Thr Val Asn Gly Glu Val Pro Thr Ala Asp Ile Thr Gly Asp
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Asp 65 Leu Ile Glu Ala Val 70 Pro Ser Leu Ala Glu 75 Lys Ala Asn Leu Asp 80

Tyr Leu Gln Val Ser Asn Val Gly Ser Asn Ser Leu Asn Tyr Thr His
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Leu Ile Pro Leu Tyr His Gly Ile Ser Glu Ala Leu Ala Ser Asp Asp
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Tyr Ala Gly Ala Val Val Thr His Gly Thr Asp Thr Met Glu Glu Thr
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Ala Phe Phe Leu Asp Leu Thr Ile Asn Ser Glu Lys Pro Val Cys Ile
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Ala Gly Ala Met Arg Pro Ala Thr Ala Thr Ser Ala Asp Gly Pro Met
145 150 155 160

Asn Leu Tyr Gln Ala Val Ser Ile Ala Ala Ser Glu Lys Ser Leu Gly
165 170 175

Arg Gly Thr Met Ile Thr Leu Asn Asp Arg Ile Ala Ser Gly Phe Trp
180 185 190

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 195 200 205

Gln Gly Tyr Leu Gly Tyr Phe Ser Asn Asp Asp Val Glu Phe Tyr Tyr
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Pro Pro Val Lys Pro Asn Gly Trp Gln Phe Phe Asp Ile Ser Asn Leu
 225 230 235 240

Thr Asp Pro Ser Glu Ile Pro Glu Val Ile Ile Leu Tyr Ser Tyr Gln
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Gly Leu Asn Pro Glu Leu Ile Val Lys Ala Val Lys Asp Leu Gly Ala
 260 265 270

Lys Gly Ile Val Leu Ala Gly Ser Gly Ala Gly Ser Trp Thr Ala Thr
 275 280 285

Gly Ser Ile Val Asn Glu Gln Leu Tyr Glu Glu Tyr Gly Ile Pro Ile
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Val His Ser Arg Arg Thr Ala Asp Gly Thr Val Pro Pro Asp Asp Ala
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Pro Glu Tyr Ala Ile Gly Ser Gly Tyr Leu Asn Pro Gln Lys Ser Arg
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28

INTERNATIONAL SEARCH REPORT

International Application No

PCT/DK 03/00684

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A23L1/03 A21D8/04 A23L1/217 A23L1/105 C12N9/82
C12N15/52

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A23L A21D C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94/28729 A (NOVONORDISK AS ; SI JOAN QI (DK)) 22 December 1994 (1994-12-22) claims 1,12,13,16,17 page 9, paragraph 1	1,4-6
X	WO 94/28728 A (NOVONORDISK AS ; SI JOAN QI (DK)) 22 December 1994 (1994-12-22) claims 1,5,11 page 8, paragraph 1	1,4-6
X	US 2002/004085 A1 (OLSEN HANS SEJR ET AL) 10 January 2002 (2002-01-10) the whole document	1,6-8

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

23 January 2004

Date of mailing of the international search report

09/02/2004

Name and mailing address of the ISA

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Vuillamy, V

INTERNATIONAL SEARCH REPORT

 Internati pplication No
 PCT/DK 03/00684

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/00029 A (NOVONORDISK AS ;WAGNER PETER (DK); NIELSEN PER MUNK (DK)) 8 January 1998 (1998-01-08) page 9, line 22 - line 31 page 6, line 29 - page 7, line 9 -----	1-5,7,9
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X	US 6 039 982 A (SI JOAN QI ET AL) 21 March 2000 (2000-03-21) column 4, line 24 - line 39 column 6, paragraph 2 - paragraph '0003! claims -----	1,2,4-6
X	DATABASE WPI Section Ch, Week 199815 Derwent Publications Ltd., London, GB; Class D11, AN 1998-162469 XP002235162 & JP 10 028516 A (KAO CORP) 3 February 1998 (1998-02-03) abstract -----	1,2,4-6
X	PATENT ABSTRACTS OF JAPAN vol. 1997, no. 05, 30 May 1997 (1997-05-30) & JP 09 009862 A (CALPIS FOOD IND CO LTD;THE;AJINOMOTO CO INC), 14 January 1997 (1997-01-14) abstract -----	1
A	"Brief Communications" NATURE, vol. 419, 3 October 2002 (2002-10-03), pages 448-449, XP002235161 USA cited in the application the whole document -----	1
A	BIEKMAN E S A: "TOEPASSING VAN ENZYMEN BIJ DE VERWERKING VAN AARDAPPELEN TOT CONSUMPTIEPRODUKTEN" VOEDINGSMIDDELEN TECHNOLOGIE, NOORDERVLIET B.V. ZEIST, NL, vol. 22, no. 20, 12 October 1989 (1989-10-12), pages 51-53, XP000069625 ISSN: 0042-7934 the whole document -----	1,4,5,7, 8
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INTERNATIONAL SEARCH REPORT

Internati application No

PCT/DK 03/00684

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 02/39828 A (DANISCO ; SOE JOERN BORCH (DK); PETERSEN LARS WEXOEE (US)) 23 May 2002 (2002-05-23) claims; example 11	1
A	K.W. KIM: "Asparaginase II of Saccharomyces cerevisiae" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 263, no. 24, 1988, pages 11948-11953, XP002266820 USA cited in the application the whole document	3

INTERNATIONAL SEARCH REPORT

Inter application No.
PCT/DK 03/00684

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/DK 03/00684

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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